

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS:

1. (previously presented) A non-painful composition of a hydrophobic protein suitable for injection in a human comprising:

- (a) a hydrophobic protein;
- (b) an amount of a zwitterionic detergent that is less than the amount required to solubilize the protein; and
- (c) an amount of a pharmaceutically acceptable nonionic detergent effective to maintain solubility of the protein in a pharmaceutically acceptable carrier and,

wherein the zwitterionic detergent is selected from the group consisting of *n*-Octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-(N,N-*n*-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate; 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; and *n*-Dodecyl-N,N-dimethylglycine.

2. (previously presented) The composition of claim 1, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

3. (previously presented) The composition of claim 1, wherein the nonionic detergent is selected from the group consisting of alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), Polyoxyethylene (20) sorbitan monolaurate, Polyoxyethylene (20) sorbitan monooleate and Polyoxyethylene (35) Lauryl Ether.

4. (previously presented) The composition of claim 3, wherein the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl).

5. (previously presented) The composition of claim 1, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate in a final concentration that is below its critical micelle concentration (CMC) and the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) in a final concentration that is above its CMC.

6. (previously presented) The composition of claim 1, wherein the hydrophobic protein is a porin.

7. (previously presented) The composition of claim 6, wherein the porin is selected from the group consisting of a gonococcal porin or a Meningococcal porin.

8. **(currently amended)** A method for producing a less-painful immunogenic composition of a hydrophobic protein in a pharmaceutically acceptable carrier suitable for administering to a mammal, comprising the steps of

(a) solubilizing said hydrophobic protein with a zwitterionic detergent to make a first composition;

(b) altering said first composition, such that the altered composition is less painful as compared to said first composition
[[and]],

wherein the altering step (b) is selected from the group consisting of (i) diluting the zwitterionic detergent where the hydrophobic protein is in a precipitated form, (ii) exchanging the zwitterionic detergent with a non-pain causing nonionic detergent, and (iii) adding a non-pain causing nonionic detergent but keeping the concentration of the zwitterionic detergent constant.

9. (previously presented) The method of claim 8, wherein the zwitterionic detergent is selected from the group consisting of *n*-Octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-(N,N-*n*-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-[(3-Cholamidopropyl) dimethylammonio]-1-

propanesulfonate; 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; and *n*-Dodecyl-N,N-dimethylglycine.

10. (previously presented) The method of claim 9, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

11. (previously presented) The method of claim 8, wherein the nonionic detergent is selected from the group consisting of alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), Polyoxyethylene (20) sorbitan monolaurate, Polyoxyethylene (20) sorbitan monooleate and Polyoxyethylene (35) Lauryl Ether.

12. (**currently amended**) The method ~~of any~~ of claim 11, wherein the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl).

13. (previously presented) The method of claim 8, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate in a final concentration that is below its CMC and the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) in a final concentration that is above its CMC.

14. (previously presented) The method of claim 8, wherein the hydrophobic protein is a porin.

15. (previously presented) The method of claim 14, wherein the porin is selected from the group consisting of a gonococcal porin or a Meningococcal porin.

16. (previously presented) The method of claim 8, wherein the zwitterionic detergent is diluted to below the critical micelle concentration.

17. (**currently amended**) A method of reducing the pain associated with administering an immunogenic composition comprising a hydrophobic protein and a zwitterionic detergent into a mammal, which method comprises

(a) altering said composition, such that the altered composition is less painful as compared to the unaltered composition, and

(b) administering said immunogenic composition ~~[[and]]~~,

wherein the altering step (a) is selected from the group consisting of (i) diluting said zwitterionic detergent where the hydrophobic protein is in a precipitated form, (ii) exchanging said zwitterionic detergent with a non-pain causing nonionic detergent, and (iii) adding a non-pain causing nonionic detergent but keeping the concentration of the zwitterionic detergent constant.

18. **(currently amended)** The method of ~~claims~~ claim 17, wherein said zwitterionic detergent is selected from the group consisting of *n*-Octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-(N,N-*n*-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate; 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; and *n*-Dodecyl-N,N-dimethylglycine.

19. **(previously presented)** The method of claim 18, wherein the nonionic detergent is selected from the group consisting of alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), Polyoxyethylene (20) sorbitan monolaurate, Polyoxyethylene (20) sorbitan monooleate and Polyoxyethylene (35) Lauryl Ether.

20. **(previously presented)** The method of claim 19, wherein the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl).

21. **(previously presented)** The method of claim 18, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate in a final concentration that is below its CMC and the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) in a final concentration that is above its CMC.

22. (previously presented) The method of claim 17, wherein the hydrophobic protein integral membrane protein.

23. (previously presented) The method of claim 22, wherein the integral membrane protein is selected from the group consisting of a gonococcal porin or a meningococcal porin.

24. (previously presented) The method of claim 17, wherein the solubility of the hydrophobic protein is maintained in said nonionic detergent.

25. (previously presented) A method of reducing the pain associated with administering an immunogenic composition comprising a hydrophobic protein and a zwitterionic detergent into a mammal, which method comprises

(a) altering said composition, such that the altered composition produces a reduction in pain as measured in the rat footpad model as compared to the unaltered composition, and

(b) administering said immunogenic composition

wherein the altered composition produces at least about a 50% reduction in pain as measured in the rat footpad model as compared to the unaltered composition.

26. (previously presented) The method of claim 25, wherein the altering step (a) is selected from the group consisting of (i) diluting said zwitterionic detergent with a non-pain causing nonionic detergent wherein the hydrophobic protein is in a precipitated form, (ii) exchanging the zwitterionic detergent with a non-pain causing nonionic detergent, and (iii) adding a non-pain causing nonionic detergent but keeping the concentration of the zwitterionic detergent constant.

27. (previously presented) The method of claim 26, wherein the altering step (a) is diluting the zwitterionic detergent with a non-pain causing nonionic detergent wherein the hydrophobic protein is in a precipitated form.

28. (previously presented) The method of claim 26, wherein the altering step (a) is exchanging the zwitterionic detergent with a non-pain causing nonionic detergent.

29. (previously presented) The method of claim 26, wherein the altering step (a) is adding a non-pain causing nonionic detergent but keeping the concentration of the zwitterionic detergent constant.

30. (previously presented) The method of any of claims 27, 28 and 29, wherein the zwitterionic detergent is selected from the group consisting of *n*-Octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-(N,N-*n*-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate; 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; and *n*-Dodecyl-N,N-dimethylglycine.

31. (previously presented) The method of any of claims 27, 28 and 29, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

32. (previously presented) The method of any of claims 27, 28 and 29, wherein the nonionic detergent is selected from the group consisting of alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl), Polyoxyethylene (20) sorbitan monolaurate, Polyoxyethylene (20) sorbitan monooleate and Polyoxyethylene (35) Lauryl Ether.

33. (previously presented) The method of any of claims 27, 28 and 29, wherein the nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl).

34. (previously presented) The method of any of claims 27, 28 and 29, wherein the zwitterionic detergent is *n*-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate in a final concentration that is below its CMC and the nonionic detergent is alpha-[4-(1,1,3,3-

tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) in a final concentration that is above its CMC.

35. (previously presented) A method of maintaining solubility of a hydrophobic protein in an immunogenic composition, which method comprises:

solubilizing a hydrophobic protein in a non-pain causing nonionic detergent, wherein non-pain causing nonionic detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl).

36. (previously presented) A method for immunizing humans with compositions containing hydrophobic membrane proteins without causing pain, which method comprises selecting alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl (Triton X-100) as a pharmaceutically acceptable detergent for maintaining solubility of hydrophobic proteins in the final formulation; wherein the concentration of Triton X-100 is above the CMC.